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Hemostatic Effects of Low-Dose Protamine Following Cardiopulmonary Bypass

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Twenty-eight patients undergoing cardiac surgery were prospectively studied and were assigned to two groups. The patients received 0.8- (Group L) or 2.0-fold (Group H) dose of protamine for the neutralization after cardiopulmonary bypass (CPB) which was determined by Hepcon HMS® assay system in which the reagent chamber containing the concentration of protamine that completely neutralized the heparin had the shortest clotting time. Mean dose of protamine was 1.60 ± 0.50 mg kg^{-1} in Group L, and 3.56 ± 1.48 mg kg^{-1}, respectively. Activated clotting times (ACT) were comparable between the two groups through this study period. In Group H, platelet counts significantly decreased to 69% of that before protamine administration, and plasma platelet factor 4 level significantly increased to approximate 2-fold of that before protamine administration just after protamine administration, respectively. However, these phenomena were not observed in Group L. In addition, these hemostatic changes occurred transiently just after protamine administration. We conclude that the low-dose protamine may prevent transient platelet depletion following CPB. Low-dose protamine can neutralize anticoagulation effect of heparin sufficiently and may mitigate protamine-induced platelet dysfunction. Am. J. Hematol. 64:112–115, 2000.

Key words: protamine; hemostasis; platelet count; platelet factor 4; cardiopulmonary bypass

Patients during cardiac and vascular surgeries received heparin to achieve systemic anticoagulation during cardiopulmonary bypass. Hemostasis at the conclusion of surgery is facilitated by neutralization of heparin with protamine. However, adverse reactions due to protamine, including hypotension, pulmonary hypertension, decrease of cardiac output, platelet dysfunction, and/or thrombocytopenia, are known [1–6].

Recently, recombinant platelet factor 4 (rPF4) was introduced for use in humans for neutralization of heparin anticoagulation and some investigators reported that rPF4 has no adverse reaction like protamine [7,8]. Nevertheless, rPF4 is not yet so popular and protamine has been still used in spite of its adverse actions.

We usually used several empirical protocols to determine the protamine dosage for the neutralization of heparin anticoagulation as follows: (1) giving a fixed amount of protamine per body weight, e.g., 3 mg/kg [9]; (2) giving protamine in a fixed ratio to total heparin dose, e.g., protamine/heparin = 1.3:1.0; (3) giving a calculated protamine dosage by a two-point heparin dose–response curve based on activated coagulation time (ACT) values measured at before and 5 min after heparin administration [10,11]. Although protocols (1) and (2) are simple, biodegradation of heparin is not considered. On the contrary, protocol (3) involved biodegradation. However ACT values are variable and inaccuracy over 400 sec [12,13]. Thus, the calculated protamine dosage may not always be optimal.

The Hepcon HMS® assay system (Hepcon HMS) (Medtronic HemoTec, Parker, CO) possesses a system of measuring heparin levels in whole blood by titration of protamine and can determine the protamine dose to be given [14,15]. Empirical protocol of protamine infusion such as 3 mg kg^{-1} is too large a dose compared with the dose determined by Hepcon/HMS. In generally, anticoagulation during CPB is managed by ACT (over 480 sec), in such a case, 3 mg kg^{-1} of protamine is equal to

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TABLE I. Patients' Characteristics*

	Group L	Group H	*P* value
Age (years old)	64.4 ± 8.7	63.4 ± 10.0	0.40
Male/female	12/2	10/4	
Weight (kg)	56.0 ± 9.3	53.7 ± 9.4	0.46
Height (cm)	161.2 ± 7.4	158.0 ± 8.4	0.43
Operation (no. of Patients)			
CABG	9	8	
AVR	5	6	
Platelet count before CPB (/μL)	171,500 ± 49,600	146,900 ± 44,500	0.18
Duration of CPB (min)	140.6 ± 70.5	123.0 ± 30.8	0.40
Total heparin (U)	20,900 ± 7,100	19,900 ± 4,900	0.69
Protamine (mg)	91.0 ± 32.2	190.1 ± 74.7	0.0025
(mg/kg)	1.60 ± 0.50	3.56 ± 1.48	0.00027

*Data are expressed as mean ± SD or number of patients. Abbreviations: CABG, aortocoronary bypass grafting; AVR, aortic valve replacement; CPB, cardiopulmonary bypass.

2.0-fold of heparin concentration approximately. Although 1.0–1.3-fold of protamine to the calculated heparin concentration is generally administrated, the ratio of heparin–protamine interaction is less in vivo since circulating platelets are collected from the whole body at bleeding site. We hypothesize that a protamine dose less than 1.0-fold that of the heparin concentration calculated by Hepcon HMS, such as 0.8-fold, can be enough to neutralize heparin.

The present study was designed to compare hemostatic changes after cardiopulmonary bypass between two differential protamine doses based on the calculation by Hepcon HMS.

MATERIALS AND METHODS

Once approval was obtained from our Institutional Review Board, 28 adult patients scheduled to undergo cardiac surgery utilizing cardiopulmonary bypass were selected arbitrarily and studied prospectively after obtaining informed consent. No patients had hepatic dysfunction, platelet abnormality, or pulmonary hypertension and if they were treated with antiplatelet or anticoagulant drugs, these drugs were discontinued more than 7 days before the operation.

The same team of perfusionists managed all cardiopulmonary bypass procedures. Cardiopulmonary bypass employed a roller pump and a membrane oxygenator. In each cases, heparin, 300 U kg^{-1}, was administrated prior to cardiopulmonary bypass and additional heparin (100 U kg^{-1}) was administrated every hour. When the ACT value that was measured every 30 min during CPB showed was greater than 480 sec, additional heparin (100 U kg^{-1}) was administered, respectively. At the termination of CPB, the heparin level in whole blood was estimated by the heparin–protamine titration by Hepcon HMS. The reagent chamber containing the concentration of protamine that completely neutralized the heparin had the shortest clotting time.

Patients were randomly assigned to a low dose (Group L) or a high dose (Group H) of protamine. At the termination of cardiopulmonary bypass, each patient in the Group L and the Group H received 0.8- or 2.0-fold dosage of protamine sulfate of heparin level estimated by Hepcon HMS. Protamine sulfate was injected to peripheral venous line over 5 min.

Each blood sample (2.8 mL) was obtained through the radial arterial canula and separated into a tube (2 mL) contained EDTA-2K for analysis of blood cell counts. The remaining of blood sample (0.8 ml) was used for measuring ACT by ACTII® (Medtronic HemoTec, Parker, CO), and heparinase–ACT was simultaneously obtained for monitoring of "heparin rebound phenomenon" [16,17]. In addition, 4 mL of blood samples were obtained from 8 patients in the both groups, respectively. The samples were put into the tubes that contained EDTA, theophylline, and dipyridamole (Diatube®; Boehringer Mannheim, CO) for analysis of plasma platelet factor 4 level. Plasma in the tube was stored at −40°C until analysis. The level of plasma platelet factor 4 was measured by enzyme immunoassay (Asserachrom PF4®; Boehringer Mannheim, CO) within a month.

Data were expressed as mean ± SD. The results of multiple groups were obtained by one-way analysis of variance for repeated measurements, and comparisons between groups were assessed by Scheffe's test. Comparisons between the groups were assessed by Student's *t*-test for unpaired data. A P value <0.05 was considered statistically significant.

RESULTS

As shown in Table I, the patients' age, the duration of cardiopulmonary bypass, and the mean total dosage of heparin were comparable between the two groups. The mean dosage of protamine in Group L was significantly less than in Group H (1.60 ± 0.50 versus 3.56 ± 1.48 mg

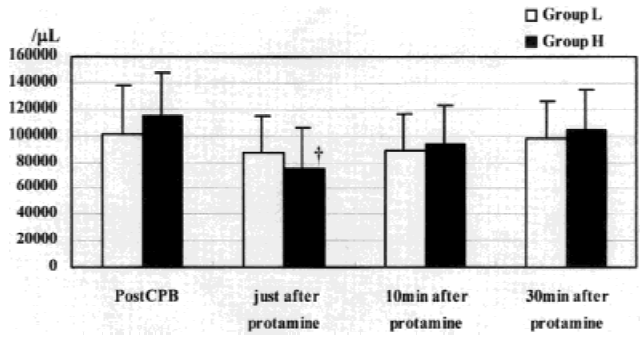

Fig. 1. Platelet changes following cardiopulmonary bypass (CPB). Platelet counts significantly decreased just after protamine administration in Group H compared with before protamine administration. Each data bar represents mean ± SD. †$P < 0.05$ compared with the platelet count of postCPB.

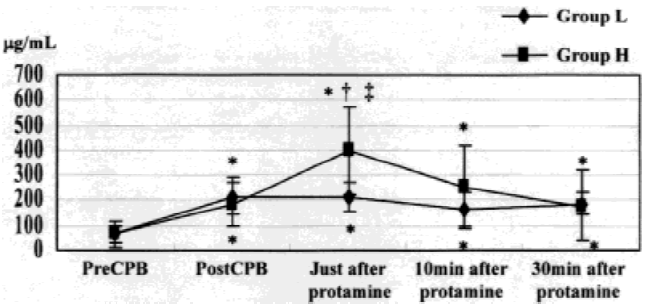

Fig. 2. Plasma platelet factor 4 (PF4) changes before and following cardiopulmonary bypass (CPB). Plasma PF4 levels significantly increased in Group H compared with in Group L just after protamine administration. Each data bar represents mean ± SD. †$P < 0.05$ compared with the value of postCPB. ‡$P < 0.05$ compared with value in Group L. *$P < 0.05$ compared with the value of preCPB.

kg^{-1}; $P = 0.00027$), although the mean dosage of protamine in Group H was similar to the dosage commonly recommended [18].

There were no episodes of severe hypotension, systolic arterial blood pressure (sABP) less than 70 mmHg, or pulmonary hypertension, or systolic pulmonary arterial pressure (sPAP) more than 40 mmHg, which was required with any inotropic or vasoconstrictive agents throughout the present study.

Blood Cell Counts

The number of platelet transiently decreased at termination of protamine administration compared with before protamine injection in Group H (Fig. 1). White blood cell counts were not different between the two groups during throughout this study period.

Plasma PF4 Level

In both groups, plasma PF4 levels increased significantly after cardiopulmonary bypass compared with before. Furthermore, plasma PF4 level in Group H temporarily, but significantly, increased following protamine administration compared with that before protamine administration ($P < 0.05$), and this level was significantly higher than in Group L. On the contrary, plasma PF4 level in Group L was almost constant after cardiopulmonary bypass (Fig. 2).

Activated Clotting Time

ACT value returned the value prior to heparin administration in all patients (Fig. 3). Furthermore, the difference between ACT and heparinase-ACT value ranged within 10 sec in 47 of 48 blood samples. Only one sample in Group H showed 20 sec of the difference (151 versus 171 sec) at 30 min after protamine administration, and an additional dose of protamine (10 mg) was given to this patient.

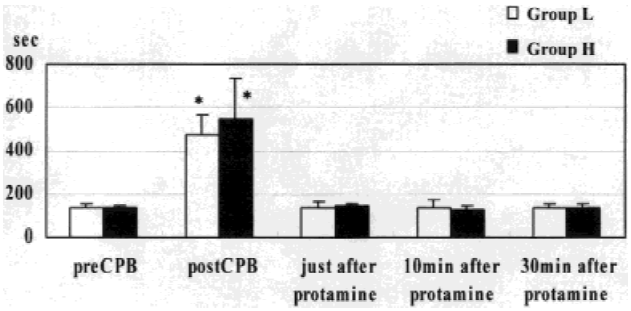

Fig. 3. Activated coagulation time (ACT) changes before and following cardiopulmonary bypass (CPB). Activated coagulation times (ACT) were not significant between the two groups throughout the study period. Each data bar represents mean ± SD. *$P < 0.05$ compared with the value of preCPB.

DISCUSSION

The present results support previous suggestions that lower doses of protamine than those commonly recommended in clinical practice can effectively neutralize heparin anticoagulation [11]. In addition, reduction of dose of protamine may prevent to impair platelet function.

ACT was not different between the two groups. Thus the effects of low doses of protamine were similar to those of high dosages with regard to neutralization of heparin-induced anticoagulation. Protamine itself includes mild anticoagulation effect [7]. However, the high dose of protamine in this study did not affect ACT. In this study, the mean protamine dosages in Group H (3.56 mg kg^{-1}) were not so large compared with the dosage commonly recommended [18]. We consider that the anticoagulative effect of protamine may be induced by much larger dose of protamine administration.

In present study, transient, but significant, decreases in platelet counts and elevation of the PF4 level were ob-

served in Group H, but not in Group L, just after protamine administration. Although similar transient thrombocytopenia after protamine administration was reported by Kirklin et al. and Bjoraker et al. [6,19], the precise mechanism of these phenomena in humans is unknown. In their studies, protamine dosages were similar to or greater than the mean dose in Group H. However, their study did not investigate the hemostatic effects of low dose of protamine in humans. In this study, the low dose of protamine could neutralize the heparin anticoagulation effect without transient thrombocytopenia and elevation of PF4 levels just after protamine administration.

A laboratory study by Velders et al. has documented that overdose protamine can induce platelet dysfunction in dogs [20]. In their experiment, excessive protamine infusion facilitates more massive intravascular platelet aggregation, resulting in a substantial decrease in the number of circulating platelet in comparison with titrated dose of protamine. In addition this protamine-induced platelet aggregation is partially reversible unlike thrombin-induced platelet aggregation which is not reversible. In the present study, we also measured PF4 level. PF4 is released from α granule of platelet and elevation of PF4 levels indicates platelet aggregation [21]. Our findings of the transient decrease of platelet and elevation of PF4 level just after protamine administration in Group H suggest that the occurrence of intravascular partial and reversible platelet aggregation following protamine administration [22]. Thus hemostasis following the high dose of protamine may be attenuated compared with the low dose of protamine.

In the present study, an additional dose of protamine was required in one case in Group H. This may be caused by reinfusion of the processed blood of the autotransfusion system suctioned from the operation field and remaining in the pump circuit.

We conclude that the dose of protamine is reduced to a minimum by heparin–protamine titration to prevent the reduction of platelet caused by protamine following cardiopulmonary bypass.

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